“Studies on Characterization Plant Growth Promoting Activity of Siderophore Producing Bacteria& Their Application for Economically imp plant”

A Synopsis for the Degree of

DOCTOR OF PHILOSOPHY IN MICROBIOLOGY

Submitted to

Swami Ramanand Teerth Marathwada University, Nanded

BY

Ms. Vidya Vijaykumar Sonalkar
M.Sc.NET (Microbiology)

RESEARCH GUIDE

Prof. Dr. S. M. More

Professor and Head

Department of Microbiology
Yeshwant Mahavidyalaya, Nanded (MS)

December -2024

Introduction

In upcoming years, the fundamental improvement of food production is the serious issue worldwide. To meet the food demands with expanded global population the farmers are mostly depend on chemical fertilizers which fulfil the demand of population but, it is increasing the risk of water pollution, human health and soil fertility. To overcome such serious problems the farm cultivation is attracted by use of plant growth promoting rhizobacteria which may be used as alternative to agrochemicals. **(Sultana *et.al*., 2021)**The PGPR are promising for sustainable farming in front of agrochemicals. The mechanism by which PGPR promote plant growth may be direct or indirect. The direct mechanism involves either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or producing secondary metabolites or modulating plant hormone levels and indirect mechanism involves decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents. The direct mechanism of producing secondary metabolite is the main area of interest in recent years. There are variety of secondary metabolites produced by PGPR which help the plant to protect from pathogens, herbivores,biotic and abiotic stresses. The environmental stresses may be temperature, pH, salinity, deficiency of micronutrients or macronutrients in soil. The micronutrients required by plants include boron, copper, iron, manganese, zinc, molybdenum, nickel etc. All these micronutrients play a vital role in plant growth. The micronutrient deficiency is increasing crises of food production in all over the world. Iron is a micronutrient for plants essential for many metabolic pathways, DNA synthesis, respiration, chlorophyll production, and photosynthesis. It is also a vital constituent of many enzymes, hence is required for a wide range of biological functions **(Sultana *et.al*., 2021)**. Plant that are iron deficient have a range of symptoms knows as chlorosis. Due to insufficient iron, it is characterized by the formation of yellow leaves. This is necessary for the proper generation of chlorophyll.Certain crops known to be sensitive to iron chlorosis are peach, kiwifruit, citrus and pear nevertheless, certain other vital crops, such as rice, soyabean, tomato and pea**. (Ahmed et.al. 2014)**

Iron is the fourth most abundant element in the Earth's crust **(Maheshwari *et.al*., 2019).** But Fe is mainly found in insoluble form as a ferric oxidation state (Fe^{3+}) which is not easily accessible to both plants and rhizospheric microorganisms**(Sultana *et.al*., 2021)**.

Siderophore production by microorganisms has received great attention due to their application in different branches of the agriculture sector such as soil science, plant

pathology and environmental sciences **(Ghazy*et.al*., 2021)**.To survive in iron deficient environment the bacteria have ability to scavenge the iron by production of siderophore **(Maheshwari *et.al*., 2019).** *Rhizobium, Arthrobacter, Azospirillum, Pseudomonas, Azotobacter, Bacillus Acinetobacter, Alcaligenes, Beijerinckia, Burkholderia*and*Enterobacter* these are the some of the examples of siderophore producing bacteria**(Maheshwari *et.al*., 2019).** The ability to synthesize and transport siderophores varies widely between microbes: some microbes can produce one or more siderophores, some cannot produce siderophores themselves but possess the siderophore uptake machinery to use exogenous siderophore-bound iron, and some cannot produce or utilize siderophores **(Park *et.al*., 2023).**The bacteria which have ability to produce siderophore they use two different mechanism of siderophore production inside the cell first by the nonribosomal peptide synthetase (NRPS) pathway and second is the NRPS-independent siderophore synthetase pathway.

These siderophores are then secreted to the environment **(Park *et.al*., 2023).** Siderophores act iron scavenger where they make a complex with Fe^{3+} on bacterial membrane, then reduces it to Fe^{2+}. This soluble form of iron is available for both themselves and plants under iron-limited conditions. After releasing of Fe^{2+} inside the cell, the siderophores are excreted and recycled for iron transport. Siderophores also play an important role in bioremediation of heavy metals from contaminated sites by binding to the toxic metals such as Cr^{3+}, Al^{3+}, Pb^{2+}, Cd^{2+}, Hg2+, etc.**(Shaikh *et.al*., 2016)**Siderophores are mainly grouped into three types: hydroxamates, catecholates, and carboxylates.Hydroxamate type of siderophore comprises linear and cyclic compound with 1-amino-5-hydroxaminopentane which binds to iron. These siderophores are not degraded by enzymatic hydrolysis or any other environmental effects due to robust binding and hexadentate structure**(Maheshwari *et.al*., 2019).** More than 500 different types of siderophores are known and out of them 270 have been structurally characterized . Most of the bacterial siderophores are catecholates (i.e. enterobactin), and some are carboxylates (i.e. rhizobactin) and hydroxamates (i.e. ferrioxamine B). Hydroxamate family is characterized by the use of one or more hydroxamic acid groups derived from amines such as lysine or ornithine to bind ferric iron. The catecholate, or catechol type, are the second most common siderophores class aside from the fact they are found to be produced by bacteria only. They contain either a mono or dihydroxybenzoic acid residue that is used to chelate ferric iron and is derived from dihydroxybenzoic acid. **(Kumar *et.al*., 2018)**

Review of Literature

➢ National status

Indian government is giving more attention towards sustainable farming by attracting farmers towards use of PGPR. PGPR plays an important role in plant growth which can be beneficial for fulfilling the healthy food requirement for Indian population. The soil profile of India is although rich in Fe concentration, the deficiency concentration is 13%. There are some states in which Fe deficiency is decreasing rapidly Gujarat (23.9%), Haryana (21.6%), Maharashtra (21.5%), newly created Telangana (17.0%) and Andhra Pradesh (16.8%) due to which threat to the crop production is also increasing.**(Shukla *et.al*., 2014)**On an average, crop responses to soil and siderophore application of Fe ranged from 0.45 to 0.89 t ha-1 for cereals, 0.30 to 0.68 t ha-1 for millets, 0.34 to 0.58 t ha-1 for pulses, 0.16 to 0.55 t ha-1 for oil seeds, 0.20 to 1.53 t ha-1 for vegetables, and 0.39 to 9.68 t ha-1 for cash and other crops .**(Shukla *et.al*., 2019)**

➢ International status

Siderophore is the indirect economical product for national and international agriculture system. It is used for several purposes in the field of agriculture such as in plant protection, crop improvement, improving soil fertility **(Ghosh *et.al*., 2020).** Worldwide, diseases of plants lead to detrimental effects on the economy and the food supplies and pose serious health hazards to both humans and animals. Due to which decrease in grain yield was observed up to 40% **(Ghazy*et.al*. 2021)**. To increase the crop yield and improving agricultural practices different countries like USA, UK, Egypt, Canada are focused to use PGPR which may help to solve such serious issues. The countries are also focusing on use of siderophore, the secondary metabolite of microorganism which help to chelate the iron in soil and made it available for plants. The use of siderophore producing bacteria showed the good results in agriculture practices by increasing crop yield.

The work done by earlier workers have been reviewed and are presented in the following paragraphs.

Ahmed *et.al* (2014) reviewed the work on siderophore and concluded that siderophores play vital role not only as iron chelator but also chelated other metals which are required by planta and bacterial cells. The iron is required for enzymatic processes, oxygen metabolism, electron transfer, and DNA and RNA synthesis. They reported that siderophore is

attracting research area because of its function as biocontrol's, biosensors, and bioremediation and chelation agents, in addition to their important role in weathering soil minerals and enhancing plant growth.

Shukla *et.al* (2014) wrote an article which states that micronutrients are required by human beings is provided through either plants or animals products. Micronutrient deficiency in soil is increasing threat about soil fertility, human health. There is decrease in micronutrient in the range of 43.0%, 12.1%, 5.4%, 5.6% and 18.3% soils are deficient in Zn, Fe, Cu, Mn and B, respectively. This article highlights current scenario of micronutrients status in Indian soils, its contribution to total food grain production and its relation to food and nutritional security of the country

Solanki *et.al* (2014) studied the effect of siderophore producing bacteria against *R. solani*, which cause damping-off and root rot in many economically important plants. They isolated the *Pseudomonas fluorescence* strain which was applied in tomato field. The result shown the strong biocontrol abilities and its application as soil mixed with seedling root treatments provided strong shield to plant roots against *R. solani* and could be used for effective bio management of pathogen

Shaikh, S. S *et.al* (2016) claimed that they only first reported the e Statistical optimization siderophore production process on laboratory bioreactor. They enhanced the production of siderophore by applying two stage statistical approach, first Plackett–Burman design and second response surface methodology (RSM) using central composite design (CCD). In first stage they studied only 3 variables pH, temperature and succinic acid. In second stage they optimized the concentration of other variables. This validation increased the siderophore yield by 6.10% while they also scale-up the siderophore production at 5L capacity reactor.

Ghazy*et.al* (2021) isolated strains of siderophore producing bacteria. The 2 potent strains *B. subtilis* and *P. koreensis* showed the good impact against late wilt disease, caused by *Cephalosporiummaydis* in maize plant. In feld experiment, mixture of *B. subtilis* and *P. koreensis* showed signifcant increases in catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO) activities, as well as total chlorophyll and carotenoids than control treatments during the two growing seasons. In the same way, the highest effect in reducing infection and increasing the thickness of the sclerenchymatous sheath layer surrounding

the vascular bundles in maize stem was observed and these results were a reflection of the increase in yield and yield parameters.

Nithyapriya *et.al* (2021) isolated the rhizospheric bacteria producing siderophore. The strain was studied for multiple plant growth promoting traits in which it had shown positive for hydrogen cyanide (HCN), ammonia, and indole acetic acid (IAA), and solubilized phosphate. In succinic acid media the total amount of siderophore produced was 296 mg/L. The characterization of siderophore revealed that it is bacillibactin siderophore. The present study concluded that the isolated strain *Bacillus sp*. LSBS2 can be used to improve iron absorption and plant growth in Sesamum indicum.

SrivastavaP*et.al* (2022) reported the first siderophore producing isolate from D. sissooRoxb. forest habitat. The isolated strain was identified as *Pseudomonas monteilii* strain MN759447. In the first-stage single-factor experiment, among the five variables studied, only pH, NH4NO3 concentration, and Fe concentration a production was carried out using a Box–Behnken design with response surface methodology. By using LC-MS, two derivatives of pseudomonine, salicylic acid, and kynurenic acid were detected as siderophores. It also exhibited a broad range of antagonistic activity against *Aspergilluscalidoustus* (65%), *Fusariumoxysporum* (41.66%), *Talaromycespinophilus* (65%), and *Talaromycesverruculosus* (65.1%) that are linked to sissoo mortality

➢ Significance of study

Over the last two decades with high technical developments use of synthetic pesticide and fertilisers is very common in agri-field for plant protection and enhancement of crop yield. This short termeconomicbenefit is acquiring the long term non profitable, non-sustainable issues regarding crop yield worldwide **(Ghosh *et.al*. 2020).**To address the emerging problem of micronutrient deficiencies in crops in mid-1960s, Indian Council of Agricultural Research (ICAR) established the All India Coordinated Scheme of Micronutrients in Soils and Plants during 1967. This project came with the study of micronutrient deficiency. It showed deficiencies of Fe increased slightly from 11% in 1967-1987 to 12.8%, respectively in 2011-2017. **(Shukla *et.al*., 2019)**

To stop this irreversible threat in agriculture the research had gave boon in the form of microorganisms. Siderophore, a product of microbes, iron chelating protein compound, is gradually acquiring a promising place in agriscience and medical science. Siderophore producing bacteria have increased attention in last two decades to improve the iron

nutrition of soil and plants. They have also been reported to slow down the growth of pathogens especially fungi by limiting the iron concentration. This is eco-friendly alternative to hazardous pesticides which is nontoxic natural product of microbes and helps to increase soil fertility, crop yields, plant protection **(Ghosh *et.al*., 2020)**

To overcome the agricultural issues chemical fertilisers can be replaced partially by use of PGPR. By understanding the importance of agricultural sciences in terms of soil health, plant growth, micronutrient deficiency, the present study will be aimed to improve the crop production, soil health and fulfilment of micronutrient to plants. Therefore, the present study has the following objective:-

OBJECTIVES

1. To isolate and screening of siderophore producing microorganisms.
2. To analyse qualitative and quantitative siderophore production by screened Microorganisms.
3. Characterization of isolate.
4. Media optimization for high yield of siderophore production
5. Lab / Green house level study of effect of potent isolates on economically important plant.

➢ Material and methods

Siderophore producing microorganisms plays the vital role in sustainable agriculture. They are increasing their importance by improving soil health, plant growth and plant protection. The study involves interaction with farmers regarding crop yield, soil profile analysis frequency and use of chemical pesticides throughout the year, economical loss due to poor crop yield. The farmers involved in this study will be from Kolhapur District, Maharashtra. Near about 50-60 farmers will be involved through different questionaries doing different economically important farming.

1. Collection of soil sample

The soil sample will be collected from the agricultural field in which crops are suffering from iron deficiency issues in soil.

2. Isolation of siderophore producing microorganisms

Isolation will be carried out by serial dilution method using nutrient agar medium. The plates will be incubated at room temperature for 24-48 hours. After incubation period the well isolated, hairy growth, submerged colonies will be selected.

3. Screening of siderophore producing microorganisms

The isolates will be screened for siderophore production using Chrome Azural S (CAS) plates. The isolates will be spot inoculated equidistance places on the plates CAS agar medium. The plates will be kept at room temperature for 24 - 48 hours. The appearance of halo zones around the colonies indicate the siderophore (SP) production.

4. Qualitative and quantitative analysis of siderophore

Qualitative analysis of siderophore will be done by CAS agar plates while quantitative analysis will be done by CAS shuttle assay.

5. Identification of functional group of siderophore

24 hours old culture supernatant will be subjected to various test for the detection of functional group. Various iron chelating functional group accessed by following test

1. Hydroxamate type of siderophore- Tetrazolium test
2. Catecholate type of siderophore – Arnow's test

6. Media optimization for improving siderophore yield

The impact of different media components such as carbon source, nitrogen source, phosphorous source, pH, temperature and salt concentration will be studied by planket-burmon or ROS method.

7. Lab / Green house level study of effect of potent isolate on economically important plant.

The potent isolates which will give highest yield of siderophore production will be further used for lab / greenhouse level study. The pot level study will be done at lab level on economically important plants of India.

8. Characterization of potent isolates.

The potent isolate will be identified by doing biochemical characterization at lab level.

To confirm the genus and species of potent isolate 16S rRNA gene will be used.

Year-wise plan of work and targets to be achieved

Sr. No.	First Year	
1	Completion of course work	At S.R.T.M.U. Nanded/ Yeshwant Mahavidyalaya, Nanded.
2	Collection of references and review of literature writing	Yeshwant Mahavidyalaya/S.R.T.M.U. Library, Nanded, Online paper access through different sites Google Scholar, Research Gate, Sci Hub.
3	Experimental trials	1.Collection of soil sample. 2.Isolation of siderophore producing microorganisms 3.Screening of siderophore producing microorganisms
Second Year		
4	Qualitative and quantitative analysis of siderophore	1. Qualitative analysis by CAS agar plate method 2. Quantitative analysis by CAS shuttle assay.
5	Identification of functional group of siderophore	1. Hydroxamate type of siderophore- Tetrazolium test 2. Catecholate type of siderophore – Arnow's test
6	Media optimization for improving siderophore yield	The impact of different media components such as carbon source, nitrogen source, phosphorous source, pH, temperature and salt concentration will be studied by Planket-Burmon or ROS method.

Third Year		
7	Lab /Green House level study of effect of potent isolate on economically important plant.	The potent isolates which will give highest yield of siderophore production will be further used for Lab /Green House level. The pot level study will be done at lab level on economically important plants of India.
8	Characterization of potent isolate.	The potent isolate will be identified by doing biochemical characterization at lab level. To confirm the genus and species of potent isolate 16S rRNA gene will be used.
9	Writing research paper National and International journals	For National and International reputed scientific peer review/UGC CARE/ /WOS/Journals
10	Submission of Final synopsis	At S.R.T.M.U. Nanded
11	Pre-PhD seminar	Yeshwant Mahavidyalaya, Nanded
12	Thesis writing and Submission	To S.R.T.M.U. Nanded

➢ REFERENCES

1) Ahmed, E., & Holmström, S. J. (2014). Siderophores in environmental research: roles and applications. *Microbial biotechnology*, *7*(3), 196-208.
2) Arora, N. K., & Verma, M. (2017). Modified microplate method for rapid and efficient estimation of siderophore produced by bacteria. *3 Biotech*, *7*(6), 381.
3) Ghazy, N., & El-Nahrawy, S. (2021). Siderophore production by *Bacillus subtilis* MF497446 and Pseudomonas koreensis MG209738 and their efficacy in controlling Cephalosporiummaydis in maize plant. *Archives of microbiology*, *203*(3), 1195-1209.
4) Ghosh, S. K., Bera, T., & Chakrabarty, A. M. (2020). Microbial siderophore–A boon to agricultural sciences. *Biological Control*, *144*, 104214.
5) Hesse, E., O'Brien, S., Tromas, N., Bayer, F., Luján, A. M., van Veen, E. M., ... & Buckling, A. (2018). Ecological selection of siderophore-producing microbial taxa in response to heavy metal contamination. *Ecology letters*, *21*(1), 117-127.
6) Kumar, P., Thakur, S., Dhingra, G. K., Singh, A., Pal, M. K., Harshvardhan, K., ... & Maheshwari, D. K. (2018). Inoculation of siderophore producing rhizobacteria and

their consortium for growth enhancement of wheat plant. *Biocatalysis and agricultural biotechnology*, *15*, 264-269.

7) Maheshwari, R., Bhutani, N., & Suneja, P. (2019). Screening and characterization of siderophore producing endophytic bacteria from Cicer arietinum and Pisum sativum plants. *J ApplBiolBiotechnol*, *7*(5), 7-14.
8) McRose, D. L., Baars, O., Morel, F. M., &Kraepiel, A. M. (2017). Siderophore production in *Azotobactervinelandii* in response to Fe-, Mo-and V-limitation. *Environmental Microbiology*, *19*(9), 3595-3605.
9) Murakami, C., Tanaka, A. R., Sato, Y., Kimura, Y., & Morimoto, K. (2021). Easy detection of siderophore production in diluted growth media using an improved CAS reagent. *Journal of Microbiological Methods*, *189*, 106310.
10) Niehus, R., Picot, A., Oliveira, N. M., Mitri, S., & Foster, K. R. (2017). The evolution of siderophore production as a competitive trait. *Evolution*, *71*(6), 1443-1455.
11) Nithyapriya, S., Lalitha, S., Sayyed, R. Z., Reddy, M. S., Dailin, D. J., El Enshasy, H. A.,&Herlambang, S. (2021). Production, purification, and characterization of bacillibactin siderophore of *Bacillus subtilis* and its application for improvement in plant growth and oil content in sesame. *Sustainability*, *13*(10), 5394.
12) Park, J., Durham, B. P., Key, R. S., Groussman, R. D., Bartolek, Z., Pinedo-Gonzalez, P., ... & Bundy, R. M. (2023). Siderophore production and utilization by marine bacteria in the North Pacific Ocean. *Limnology and Oceanography*, *68*(7), 1636-1653.
13) Perry, W. J., Spraggins, J. M., Sheldon, J. R., Grunenwald, C. M., Heinrichs, D. E., Cassat, J. E., ... & Caprioli, R. M. (2019). *Staphylococcus aureus* exhibits heterogeneous siderophore production within the vertebrate host. *Proceedings of the National Academy of Sciences*, *116*(44), 21980-21982.
14) Prasad, K. (2022). Utilization of Siderophore Producing Plant Growth Promoting Rhizobacteria to Improve Crucial Nourishment and Management of Phytopathogen in Cash Crops for Sustainable Development. *American Journal of Sciences and Engineering Research*, *5*(2), 15-23.
15) Rizzi, A., Roy, S., Bellenger, J. P., & Beauregard, P. B. (2019). Iron homeostasis in *Bacillus subtilis* requires siderophore production and biofilm formation. *Applied and Environmental Microbiology*, *85*(3), e02439-18.

16) Santos, S., Neto, I. F., Machado, M. D., Soares, H. M., & Soares, E. V. (2014). Siderophore production by *Bacillus megaterium*: effect of growth phase and cultural conditions. *Applied biochemistry and biotechnology*, *172*, 549-560.

17) Schalk, I. J. (2024). Bacterial siderophores: diversity, uptake pathways and applications. *Nature Reviews Microbiology*, 1-17.

18) Shaikh, S. S., Wani, S. J., & Sayyed, R. Z. (2016). Statistical-based optimization and scale-up of siderophore production process on laboratory bioreactor. *3 Biotech*, *6*(1), 69.

19) Sharma, S. K., Singh, U. B., Sahu, P. K., Singh, H. V., & Sharma, P. K. (2021). *Rhizosphere microbes*. Singapore: Springer.

20) Shukla, A. K., & Behera, S. K. (2019). All India coordinated research project on micro-and secondary nutrients and pollutant elements in soils and plants: Research achievements and future thrusts. *Indian Journal of Fertilisers*, *15*(5), 522-543.

21) Shukla, A. K., Tiwari, P. K., & Prakash, C. (2014). Micronutrients deficiencies vis-a-vis food and nutritional security of India. *Indian J. Fert*, *10*(12), 94-112.

22) Singh, A. K., Upadhyaya, A., Kumari, S., Sundaram, P. K., & Jeet, P. (2020). Role of Agriculture in making India $5 trillion Economy under Corona Pandemic Circumstance: Role of agriculture in Indian economy. *Journal of AgriSearch*, *7*(2), 54-58.

23) Sinha, A. K., Parli Venkateswaran, B., Tripathy, S. C., Sarkar, A., & Prabhakaran, S. (2019). Effects of growth conditions on siderophore producing bacteria and siderophore production from Indian Ocean sector of Southern Ocean. *Journal of basic microbiology*, *59*(4), 412-424.

24) Solanki, M. K., Singh, R. K., Srivastava, S., Kumar, S., Kashyap, P. L., Srivastava, A. K., & Arora, D. K. (2014). Isolation and characterization of siderophore producing antagonistic rhizobacteria against *Rhizoctoniasolani*. *Journal of Basic Microbiology*, *54*(6), 585-597.

25) Srivastava, P., Sahgal, M., Sharma, K., Enshasy, H. A. E., Gafur, A., Alfarraj, S., ... & Sayyed, R. Z. (2022). Optimization and identification of siderophores produced by *Pseudomonas monteilii*strain MN759447 and its antagonism toward fungi associated with mortality in Dalbergia sissoo plantation forests. *Frontiers in Plant Science*, *13*, 984522.

26) Sultana, S., Alam, S., & Karim, M. M. (2021). Screening of siderophore-producing salt-tolerant rhizobacteria suitable for supporting plant growth in saline soils with iron limitation. *Journal of Agriculture and Food Research*, *4*, 100150.

27) Ul-Rehman, S., Selvaraj, M., & Syed, I. M. (2012). Indian agricultural marketing-A review. *Asian Journal of Agriculture and Rural Development*, *2*(1), 69-75.

28) Vadivelu, A., & Kiran, B. R. (2013). Problems and prospects of agricultural marketing in India: An overview. *International journal of agricultural and food science*, *3*(3), 108-118.

29) Vindeirinho, J. M., Soares, H. M., & Soares, E. V. (2021). Modulation of siderophore production by *Pseudomonas fluorescen*s through the manipulation of the culture medium composition. *Applied Biochemistry and Biotechnology*, *193*, 607-618.

30) Yu, S., Teng, C., Bai, X., Liang, J., Song, T., Dong, L., ... & Qu, J. (2017). Optimization of siderophore production by *Bacillus sp.* PZ-1 and its potential enhancement of phytoextration of Pb from soil. *Journal of microbiology and biotechnology*, *27*(8), 1500-1512.

Research scholar
Ms. VidyaVijaykumarSonalkar

Research Guide
Prof.Dr.S.M.More

Date: - / /2024

Place:-Nanded